The specification has been amended to include capitalization and generic descriptions of products identified by their trademark, as required by the Examiner at page 2 of the Office Action dated October 1, 2001 (Paper No. 7).

The claims have been amended to obviate the claim objections noted on pages 2-3 of Paper No. 7. Withdrawal of the claim objections is requested.

To the extent not obviated by the above, the Section 112, second paragraph, rejection of claims 1-29 and 41-53 is traversed. The claims have been amended to obviate the objections noted in paragraphs 13-15, 17 and 19-23 of Paper No. 7. The applicants note however, with regard to the objections stated in paragraphs 16 and 18 of Paper No. 7, that claim 15, for example, is dependent on claim 14 which is dependent on claim 12 which is ultimately dependent on claim 1 such that the recited agarose of claim 15 is submitted to find support, at least, in claim 1, from which it ultimately depends. Similarly, claim 45, which is believed to be the subject of the objection recited in paragraph 18, is dependent on claim 41, which is believed to provide appropriate antecedent basis for the recited agarose. Withdrawal of the Section 112, second paragraph, rejection of claims 1-29 and 41-53 is requested.

The Section 102 rejection of claims 17, 18, 20-23, 25, 26, 41-45, 51-52 over Kapp (Analytical Biochemistry, Vol. 206:293-299 (1992)) or Colote (Analytical Biochemistry, Vol. 154:15-20 (1986)) is traversed. Reconsideration and withdrawal of the rejection are requested in view of the following distinguishing remarks.

The Examiner is urged to appreciate that there is a well known distinction between reverse phase chromatography/reverse phase media and hydrophobic

interaction media/hydrophobic interaction chromatography and that there exists a lack of predictability between reverse phase and hydrophobic interaction techniques for macromolecules. These two points form an important basis of the distinction between the presently claimed invention and the cited art.

Specifically, there is a fundamental difference between hydrophobic interaction chromatography media (referred to as hydrophobic interaction media in the present claims) used in this invention and the reverse phase media used by Kapp et al and Colote et al (and much of the cited art). An important difference between these media is the ligand density, which has significant impact on the nature of its interaction with macromolecules. The two distinct types of chromatographic separations, namely reverse phase chromatography and hydrophobic interaction chromatography, utilize different media, namely the reverse phase media and the hydrophobic interaction media. The pending claims recite the use of hydrophobic interaction media to clearly distinguish the currently claimed invention from previous methods described by Kapp et al and Colote et al.

In addition, the method used by Kapp et al uses 1) an analytical HPLC column, the results from which cannot be extrapolated to preparative purifications; 2) non-aqueous solvents, which cannot be readily used in large scale manufacturing; 3) DMSO, which cannot be used in any process for use in gene therapy for humans; 4) reverse phase media, rather than hydrophobic interaction media; and 5) preparation methods for the starting material that can significantly alter the separation conditions, and hence severely limit the usefulness of the data in predicting selectivities in other methods.

One ordinarily skilled in the art would have predicted, based on these results, that weaker binding conditions than that described should be used for C4 /C5 ligands, whereas the current invention identified 3 M ammonium sulfate, which is a much stronger binding condition than would have been predicted.

Kapp & Colote et al's studies could not have provided any insight into designing the presently claimed process. More importantly, with regard to the Section 102 rejection, the cited art fails to teach each and every aspect of the presently claimed invention. Withdrawal of the Section 102 rejection of the noted claims over Kapp or Colote is therefore requested.

The Section 102 rejection of claims 17-23, 25, 30, 31, 34-37, 41-45 and 51-53 over McNeilly (U.S. Patent No. 6,214,586) is traversed. Reconsideration and withdrawal of the rejection are requested in view of the following distinguishing remarks.

US 6,214,586 does not teach a method for separating and isolating supercoiled plasmid DNA from relaxed plasmid DNA. Moreover, the method which is described therein is based on the use of reverse phase media, employing non-aqueous solvents, whereas the presently claimed invention provides a method for separation of supercoiled from relaxed plasmid DNA, utilizing hydrophobic interaction media, without employing non-aqueous solvents. This invention further requires an anion exchange chromatography step followed by reverse phase method using reverse phase media, whereby the plasmid DNA binds to the media.

Withdrawal of the Section 102 rejection of the noted claims over McNeilly is requested as the cited document fails to teach each and every aspect of the presently claimed invention.

The Section 102 rejection of claim 53 over Lee (U.S. Patent No. 6,197,553) is traversed. Reconsideration and withdrawal of the rejection are requested in view of the following distinguishing remarks.

The applicants respectfully submit that US 6,197,553 does not teach an effective method for endotoxin reduction. The level of reduction is significantly less than the presently claimed invention. It is known that several matrices bind endotoxin, but the current invention provides the highest binding capacity per mL of resin, while separating all other contaminants from plasmid DNA. To reiterate, Table 1 under column 1 in US 6,197,553 shows the endotoxin data from the multi-gram run. The table below shows the calculation of binding capacity from that data.

	Plasmid DNA amount	Endotoxin	Total endotoxin
Input into reverse phase	4000 mg	1.2 x 10 e 4 EU/mg	4.8 x 10 e 7
Output from reverse phase	2300 mg	62	1.426 x 10 e 5

Total endotoxin removed = Endotoxin Input into reverse phase — Endotoxin Output from reverse phase
Endotoxin removal capacity of resin = Total endotoxin removed / volume of resin
Volume of resin = 7000 mL
Endotoxin capacity = 6851 EU/mL of resin

The capacity is 6851 EU/mL of resin compared to 1 to 3 million EU/ml resin obtained with the presently claimed invention, with the added advantage of operating

the column in the flow through mode, resulting in significant reduction in economics, demonstrates that the cited document fails to teach the claimed invention. US 6,197,553 did not teach an effective or selective removal of endotoxin from plasmid DNA. The use of reverse phase media is ineffective in removal of endotoxin, while the use of hydrophobic interaction media (used by the presently claimed invention) is extremely effective. The applicants further note that industry standard for gene therapy grade materials is 10 EU/mg and therefore this method does not teach one to isolate plasmid DNA of gene therapy quality as disclosed in the background (column 2) and summary description (Column 3, line 5-6) of the cited patent.

Withdrawal of the Section 102 rejection of claim 53 is requested.

The Section 103 rejection of claims 1-53 over Lee, Maitra (Journal of Clinical Microbiology, Vol. 13 (1):49-53, Jan. 1981), Colote, Kapp, Ishida (Kagaku Kogaku Rondunshu, Vol. 17 (3):589-594, 1991), Gjerde (U.S. Patent No. 6,265,168) and McNeilly, is traversed. Reconsideration and withdrawal of the rejection are requested in view of the following distinguishing comments.

Initially, the applicants note the Examiner has combined seven references to allegedly establish a *prima facie* case of obviousness. The large number of references required by the Examiner would appear to support the applicants' belief that the claimed invention is patentable over the cited art.

Substantively, the Examiner is requested to consider the following and withdraw the Section 103 rejection in view thereof.

The applicants submit that the presently claimed invention does not provide a

method of separating supercoiled plasmid DNA using reverse phase chromatography and hence all of the Examiner's comments are not relevant. The claimed invention provides a hydrophobic interaction chromatography method using hydrophobic interaction media for plasmid DNA purification. Attached are copies of Amersham Pharmacia' s catalog that lists reverse phase media and hydrophobic interaction media under separate headings with distinct properties having been provided to distinguish between the reverse phase media and hydrophobic interaction media.

As discussed above, US 6,197,553 did not teach an effective method for endotoxin removal.

Maitra et al have shown endotoxin binding to the various matrices, but did not teach the separation of plasmid DNA from all the contaminants, namely endotoxin, RNA, protein, etc. The presently claimed invention provides, for the first time, an identification of conditions that cause all the contaminants to bind in high capacities, while flowing through the product. Identifying such conditions involves not only the understanding of the binding chemistry of one component, but an understanding of the binding of all the components, namely RNA, protein, plasmid DNA and endotoxin. None of the cited art suggests or teaches the binding characteristics of all these molecules relative to one another on hydrophobic interaction matrices. In addition, the mechanism of binding of all these macromolecules to hydrophobic interaction matrices is poorly understood in the cited art. Hence, it would not have been obvious to one of ordinary skill in the field to be able to predict from the teachings of the cited references that a method for separation of plasmid DNA from the contaminants could have been made.

Maitra et al's demonstration of endotoxin binding to hydrophobic interaction media does not teach or suggest a method for separating plasmid DNA from endotoxin, RNA, protein and other contaminants.

Ishida et al have shown endotoxin removal from antibiotics and determined capacities for endotoxin at various salt concentrations. However, they do not show how endotoxin can be separated from complex macromolecules such as plasmid DNA. As mentioned above, there is no information in the cited art of how endotoxin separates relative to RNA, protein, and plasmid DNA, which are important for the claimed invention. Identification of the relative placement of separation of these molecules on a hydrophobic interaction media to provide separation without the use of non-aqueous solvents is an important aspect of the claimed invention. Ishida et al's studies are applicable only to separation of endotoxin from antibiotics. It is well known that antibiotics behave completely differently from plasmid DNA on hydrophobic interaction media however the identification of the same does not make the presently claimed invention obvious.

The applicants have noted above the deficiencies of Kapp and Colote. The additional references cited under Section 103 would not have cured the deficiencies of Kapp or Colote or motivated one of ordinary skill in the art to have altered the references as would have been required to make the presently claimed invention.

US 6,265,168 teaches separation of DNA fragments using reverse phase media, using non-aqueous solvents, at extremely small scales. None of the teachings provide insight into the following: 1) how plasmid DNA (supercoiled & relaxed) interact with

hydrophobic interaction media under aqueous conditions; 2) how RNA interacts with HIM; 3) how this method can be used for purification of plasmid DNA for such applications as gene therapy; and 4) whether the method can be scaled. Moreover, it would have been apparent to one of ordinary skill in the art from the description provided that the method was not feasible for large scale separations as it 1) involves use of non-scalable devices; 2) uses solvents; and 3) involves resolution not achievable at large scales. One of ordinary skill could not have predicted that the method claimed in this invention, which is a scaleable process for plasmid DNA purification, in contrast to separation and analysis technique taught by US 6,265,168, could have been made, from the cited art.

US 6,214,586 does not teach a method of separating RNA from plasmid DNA. Column 4 clearly states that the RNA is removed through precipitation and not through the reverse phase chromatography method. US 6,214,586 does not teach a method for separating supercoiled plasmid DNA from relaxed plasmid DNA. There is no mention or evidence of the same in the entire patent. US 6,214,586 does not teach or suggest a method of removing endotoxin using reverse phase chromatography. There is no evidence for effective endotoxin removal in the examples. It was shown through analysis of data from another reverse phase method (US 6,197,553) that it was ineffective in reducing endotoxin levels. US 6,214,586 utilizes a series of steps for purification, including an ion exchange column, which has been shown to result in reduction of endotoxin. US 6,214,586 did not teach a method for separating genomic DNA from plasmid DNA using reverse phase chromatography. The applicants believe

that it is clear from the text and the claims that the genomic DNA was removed through the precipitation step. US 6,214,586 does not teach or suggest methods that relate to the presently claimed invention.

The cited references demonstrate that there was no consistency in the results from each of the studies presented. In addition, all of the cited references only teach separations on reverse phase media. It is well known that separation of molecules on reverse phase media is significantly different from the behavior on hydrophobic interaction media (See table in attached reference of Ziad el Rassi et al, Chromatography of Peptides and Proteins, pp. 447-494).

The Examiner contends that the claimed invention utilizes reverse phase chromatography, which is inaccurate. The claimed invention provides hydrophobic interaction chromatography using hydrophobic interaction media. Reverse phase chromatography and hydrophobic interaction chromatography techniques, are similar only in that they share the concept of decreasing hydrophobicity in the elution of molecules. The similarity however is limited to this aspect. The techniques, the behavior, selectivity, scalability, resolution, and integrity of molecules separated, for example, are significantly different between reverse phase and hydrophobic interaction chromatography and hence two distinct disciplines of separation exists. It has been well documented in literature that molecules separated by reverse phase chromatography using reverse phase media do not separate similarly on hydrophobic interaction media using hydrophobic interaction chromatography. Classic examples include the use of reverse phase methods for identifying and separating misfolded and partially oxidized

protein forms, whereas these forms cannot be separated on hydrophobic interaction media. It is relevant to note that one important shortcoming of the reverse phase methods is that the macromolecules undergo denaturation under the conditions of binding and elution, resulting in separations that produce unusable products. Moreover, while separation of small molecules on reverse phase media has been successfully used both as an analytical method and purification methods, it was well known that these molecules cannot be separated using hydrophobic interaction media/hydrophobic interaction chromatography. Therefore, one of ordinary skill would clearly have known that these methods are distinct and have been developed independently of each other through scientific studies. That is, the Examiner's reliance on references teaching reverse phase methods would not be considered relevant by one of ordinary skill in the art to the presently claimed invention.

On page 10, of Paper No. 7, the Examiner states "Kapp et al each taught ... using well known and obvious methods of reverse phase chromatography with hydrophobic interaction media". This is inaccurate as reverse phase chromatography taught by the authors of the cited art used reverse phase media that is distinct from hydrophobic interaction media of the presently claimed invention. Another distinction, which was well known to those ordinarily skilled in the field is that reverse phase chromatography and hydrophobic interaction chromatography techniques significantly differ in the binding and elution methods. While hydrophobic interaction chromatography focuses, for example, on binding using the hydrophobic patches that are present on the surface of macromolecules, reverse phase chromatography invariably results in

unraveling of the macromolecule to expose the hidden hydrophobic parts of the molecule, which are used for the binding. In eluting the macromolecules from the reverse phase media, invariably non-aqueous solutions are necessary due to the inherent nature of the binding interaction, while aqueous solutions with reduced salt concentrations are sufficient in eluting the macromolecules in hydrophobic interaction chromatography. These distinctions clearly indicate or demonstrate a recognized distinction.

A person of ordinary skill in the art would not have reasonably expected to use the teachings of Maitra et al and Ishida et al, or any of the cited art, to separate plasmid DNA from endotoxin at high capacities. In addition, none of the cited art describes or suggests conditions necessary, such as relative binding and elution conditions of plasmid DNA, RNA, protein and endotoxin on the hydrophobic interaction media, to make and use the presently claimed method for plasmid DNA purification.

In view of the above and attached, withdrawal of the Section 103 rejection is requested.

The claims are submitted to be in condition for allowance and a Notice to that effect is requested. The Examiner is requested to contact the undersigned if anything further is required in this regard.

Respectfully submitted,

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MARKED UP SPECIFICATION AND CLAIMS

Pages 18 to 19, delete the paragraph spanning page 18, line 19 through page 19, line 19, and substitute the following therefor:

--Preferred hydrophobic interaction media which may be used in the methods of the present invention include hydrophobic interaction chromatography resins that, for example, contain methacrylate polymer or copolymer backbones, such as methacrylate /ethylene glycol and/or methacrylate/propylene glycol copolymers (TosoHaas, Montgomeryville, PA), and/or an agarose or [Sepharose] SEPHAROSE (an agarose based chromatography matrix commercially available from Amersham Biosciences) (Amersham Pharmacia Biotech, Piscataway, NJ), such as crosslinked or non-crosslinked, agarose, [Sepharose] SEPHAROSE, dextran, silica containing polymer, organic polymers (natural or synthetic), a ceramic-containing, or a gel matrix, backbone, or a combination of any of these, with C_3 to C_{10} alkyl, branched or straight, pendent side chain ligands. Preferred pendent ligands include propyl, butyl, hexyl and/or octyl ligands. These ligands provide the preferential binding interaction which is exploited in the separation, purification and/or isolation methods of the present invention. One of ordinary skill in the art will appreciate that hydrophobic interaction resins may include ligands in addition to or in place of these alkyl ligands, which will also be useful in the method of the present invention. Examples of such ligands include, but are not limited to, phenyl, octyl, butyl, propyl, neopentyl, hydroxypropyl, benzyl, octadecyl, diphenyl, and methyl as well as substituted and unsubstituted derivatives of same, and combinations thereof. Suitable resin or media materials useful in the present invention

include those described, for example, in EP Patent No. 964057, EP Application No. 99109441, JP 2000035423, JP 99127700 and JP 98127665 (Kitamura et al.) the entire contents of each of which are hereby incorporated by reference.--

Page 20, delete the paragraph spanning lines 9-14 and insert the following therefor:

-- The beads used in ["Streamline" (Amersham Pharmacia biotech)] STREAMLINE (Chromatography column, including media available from Amersham Biosciences for expanded bed adsorption separation) columns typically are larger in size with different densities, and are made by various manufactures, including Amersham Pharmacia Biotech, Biosepra Inc, but not limited to these, where the clarified lysate could be flowed through these "expanded bed" columns, resulting in removal of contaminants through binding to the beads that contain the hydrophobic interactive ligands.--

Pages 21 to 22, delete the paragraph spanning page 21, line 21 through page 22, line 5 and insert the following therefor:

-- Figure 4 (insert) shows a scanned image of an agarose gel from Example 5 (butyl HIC) stained with SYBR GOLD (Stain available from Molecular Probes for staining nucleic acids on agarose gels) wherein lane 1 contains a supercoiled DNA Ladder; lanes 2 and 3, contain samples from peak 1 (relaxed) and lanes 3-5 contain samples from peak 2 (supercoiled). (Lanes being numbered from left to right.) The chromatogram from Example 5 of absorbance versus volume shows the separation of relaxed (peak 1) and supercoiled DNA (peak 2).--

Page 28, delete the paragraph spanning lines 1-16 and insert the following therefor:

--An attractive feature of this method of endotoxin removal is the immense capacity of the resin for the endotoxin, of approximately 1,000,000 EU/ml of resin, in addition to the simplicity and >95% recovery of plasmid DNA. For example, a plasmid DNA solution containing 500 mg of plasmid and 10 million EU of endotoxin can be purified using 10 ml of resin, whereas, at least 1,000 to 4,000 ml of an anion exchange resin would be required for binding the plasmid DNA and the endotoxin, with the added disadvantage of poor recoveries on such an anion exchange resin. The method of the present invention therefore results in savings of 100 to 400 fold in resin cost, and additional savings on column cost and increased recovery of product. The commercially available DNA Etox resin is currently at least 8 fold more expensive than the resins used in the method of the present invention. Another commercial resin ([PolyFlo] POLY-FLO (Chromatography media available from PureSyn Inc. for plasmid DNA purification) – PureSyn Inc., 87 Great Valley Pkwy Malvern, PA 19355) with proprietary chemistry that is useful in endotoxin removal is 5 to 10 fold more expensive and requires the use of solvents and ion-pairing chemicals.--

Pages 32 to 33, delete the paragraph spanning page 32, line 17 through page 33, line 13 and insert the following therefor:

--A Butyl 650S column (Butyl 650S resin from TosoHaas Inc., 156 Keystone Drive, Montgomeryville, PA 18936) of 2.6 cm diameter and 15 cm bed height, of approximately 75 ml bed volume was packed and equilibrated with TE buffer, pH 7.4,

containing 2M AS. The sample was loaded at a flow rate of 5 ml/min. The flow through was collected, and samples were taken for analysis (DNA concentration, agarose gel, and endotoxin assay. Endotoxin assay was performed with spikes and samples were diluted appropriately to obtain PPC (Positive Product Control) recoveries in the range considered acceptable. Endotoxin concentrations were determined using the BioWhittaker [Kinetic-QCL] KINETIC-QCL (Endotoxin assay kit available from BioWhittaker) Chromogenic LAL assay as described in BW publication No. P50-650U-5, [Kinetic] KINETIC-QCL Test Kit Manual. Following the sample load, TE containing 2M ammonium sulfate was flowed through the column, and collected and sampled. The column was subsequently washed with TE buffer - pH 7.4, USP purified water, and cleaned with 0.5N sodium hydroxide, and rinsed with >15 volumes of USP purified water. Endotoxin was present in each of these washes as shown below in Table 1. In addition to this outstanding endotoxin removal efficiency, significant amount of RNA, protein, and DNA fragments were removed, leaving the sample significantly purified.--

Page 46, delete the paragraph spanning lines 1-15 and insert the following therefor:

-- *E. coli* cells harboring the plasmid pE1A-K2 was grown, and lysed using chemical methods, and clarified through centrifugation methods. The supernatant was used for the experiment. The sample was purified through an anion exchange column ([Q Hyper D] Q-HYPER D (Anion exchange chromatography media) – BIOSEPRA Inc.). A 2M sodium chloride elution from the column was used for this experiment. The sample was present in 50mM Tris 10mM EDTA pH 7.4 buffer with 2M NaCl. A Butyl

HIC column (using Butyl 650S resin – TosoHaas) of diameter 1 cm and height 20 cm of approximately 10ml volume was packed and equilibrated with TE containing 2M sodium chloride. The sample was loaded at a flow rate of 2 ml/min. The flow through was collected, and samples were taken for analysis (DNA concentration, agarose gel, and endotoxin assay). Following the sample load, TE containing 2M ammonium sulfate was flowed through the column, collected and sampled. The column was subsequently washed with TE pH 7.4.--

IN THE CLAIMS

1. (Amended) A method for purifying plasmid DNA from a mixture of same containing at least one host cell impurity comprising the following steps:

(a) forming a solution by adding sufficient salt to said mixture to allow selective binding of said at least one host cell impurity to a hydrophobic interaction media;

(b) contacting said solution containing plasmid DNA with said hydrophobic interaction media under conditions that said at least one impurity binds to the hydrophobic interaction media to form a complex; and

(c) collecting unbound plasmid DNA from said complex [and hydrophobic interaction media];

wherein said method is conducted in the absence of non-aqueous solvents, detergents, glycols, hexamine cobalt, spermidine, and polyvinylpyrollidone.

5. (Amended) The method of claim 4 wherein the salt is ammonium sulfate in a concentration range of about 2M to 4M.

7. (Amended) The method of claim 1 wherein the solution comprises sodium salts in a concentration range of about 2M to 4M.

14. (Amended) The method of [claims] claim 12 wherein the hydrophobic interaction media are selected from the group consisting of a methacrylate polymer or copolymer backbone bound to a least one of a propyl, butyl, hexyl, octyl, nonyl or decyl ligand.

16. (Amended) The method of claim 12 wherein the [resin] support is in the form of bead in the size range of 15 to 100 μm.

17. (Amended) A method of separating supercoiled plasmid DNA from a mixture of supercoiled plasmid DNA and relaxed plasmid DNA and, optionally, at least one host cell impurity comprising the following steps:

(a) forming a solution by adding a salt to the mixture of supercoiled plasmid DNA and relaxed plasmid DNA and, when present, said at least one host cell impurity;

(b) contacting the solution with a hydrophobic interaction media under a first [conditions] condition where both the supercoiled plasmid DNA and relaxed plasmid DNA bind to the hydrophobic interaction media to form a bound first mixture;

(c) altering the first [conditions] condition surrounding the bound first mixture to a second [conditions] condition to remove relaxed plasmid DNA from the bound first mixture to form separate components containing a second bound mixture and relaxed plasmid DNA; and

(d) modifying the second [conditions] condition surrounding the said [bound] second bound mixture to a third [conditions] condition to remove supercoiled plasmid

DNA from said second bound mixture to form separate components containing hydrophobic interaction media and supercoiled plasmid DNA.

27. (Amended) The method of claim 17 wherein the first [conditions] condition comprises equilbrating said media with a salt solution containing ammonium sulfate which is present in a concentration range of about 2.5M to 4M.

28. (Amended) The method of claim 17 wherein the second [conditions] condition comprises washing the media with a salt solution containing ammonium sulfate in a concentration of about 2.35M to about 2.45M.

29. (Amended) The method of claim 17 wherein the said third [conditions] condition comprises washing said second bound mixture with a salt solution containing ammonium sulfate in a concentration of about 1M to 2.3M.

41. (Amended) A method of separating supercoiled plasmid DNA from relaxed plasmid DNA comprising contacting a mixture of supercoiled plasmid DNA and relaxed plasmid DNA with a hydrophobic interaction media under a first [conditions] condition where both the supercoiled plasmid DNA and the relaxed plasmid DNA bind to said hydrophobic interaction media to form a bound first mixture, altering said first [conditions] condition surrounding the bound first mixture to a second [conditions] condition to remove said relaxed plasmid DNA from said bound first mixture to form separate components containing a second bound mixture and said relaxed plasmid DNA, and modifying the second [conditions] condition surrounding said second bound mixture to a third [conditions] condition to remove said supercoiled plasmid DNA from

said second bound mixture to form separate components containing said hydrophobic interaction media and said supercoiled plasmid DNA.

46. (Amended) The method of claim 41 wherein said [resin] support is in the form of beads ranging in size from 35 to 100 μm.

47. (Amended) The method of claim 41 wherein said first [conditions] condition comprises equilibrating said [mixture and] media with a salt solution containing ammonium sulfate in a concentration range of about 2.5 M to about 4 M.

48. (Amended) The method of claim 47 wherein said second [conditions] condition comprises washing said bound first [bound] mixture with a salt solution containing ammonium sulfate in a concentration of about 2.35 M to about 2.45 M.

49. (Amended) The method of claim 48 wherein said third [conditions] condition comprises washing said second bound mixture with a salt solution containing ammonium sulfate in a concentration of about 1 M to about 2.3M.

52. (Amended) A method for the enriching [the amount of] supercoiled DNA relative to relaxed DNA in a mixture thereof, the method comprising:

(1) interacting [the] a mixture containing supercoiled DNA and relaxed DNA with a hydrophobic interactive media comprising an alkyl moiety under ionic conditions wherein the supercoiled DNA preferentially binds to the hydrophobic interactive media;

(2) treating the hydrophobic interactive media [containing the], relaxed DNA and supercoiled DNA under ionic conditions that allow the preferential removal of the relaxed DNA; and

(3) eluting the supercoiled DNA from the hydrophobic interactive media.

53. (Amended) A method for removing lipopolysaccharide (LPS) from a composition containing DNA, the method comprising:

(1) interacting [the] a mixture comprising the DNA and LPS with a hydrophobic interactive media comprising an alkyl moiety, wherein the interacting is under ionic conditions where the LPS preferentially binds to the hydrophobic interactive media relative to the DNA; and

(2) treating the hydrophobic interactive media containing the DNA and LPS with ionic conditions that allow the selective removal of the DNA.